REMARKS

Applicants submit herewith a translation of JP Publication No. 06-205695. Although Applicants take the position that submission of a translated document is not necessary to meet the requirements of 37 C.F.R. § 1.98(a)(3) as discussed on page 4 of the Amendment filed April 5, 2005, in an abundance of caution, Applicants submit herewith a translation of JP Publication No. 06-205695 as Attachment A.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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(54) MONOCLONAL ANTIBODY COMBINING WITH BASOPHIL, SEPARATION OF BASOPHIL, RELEASE OF CHEMICAL MEDIATOR FROM BASOPHIL, AND TEST FOR RELEASING CHEMICAL MEDIATOR ORIGINATED FROM BASOPHIL

(57)Abstract:
PURPOSE: To obtain the novel antibody useful for separating basophils suitable for the releasing test of a specific chemical mediator through IgE.
CONSTITUTION: This monoclonal antibody is e.g. a monoclonal antibody BA 101, recognizing a basophil, holding its activity after immobilized on a carrier, not inhibiting the specific release of histamine through the IgE from the basophil, and not inducing the non-specific release of histamine from the basophil. The exemplified anitibody is produced with a hybridoma BA 101

Shionogi (FERM BP-4004).

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CLAIMS

[Claim(s)]
[Claim 1] The monoclonal antibody which has the following properties.
(1) Basophilic leucocyte and (2) which react (3) to which after the formation of support solid phase holds antibody activity (4) which does not check the specific histamine isolation through IgE of this basophilic leucocyte [Claim 2] which does not induce nonspecific histamine isolation of this basophilic leucocyte The monoclonal antibody according to claim 1 characterized by the class of a mouse immunoglobulin belonging to IgG1 or IgM.
[Claim 3] The monoclonal antibody according to claim 1 chosen from the group which consists of monoclonal antibodies BA101, BA20, BA135, and BA312 produced by hybridoma BA101 Shionogi (FERM BP-4004), BA20 Shionogi (FERM BP-4005), BA135 Shionogi (FERM BP-4006), and BA312 Shionogi (FERM BP-4007), respectively.
[Claim 4] The solid phase-ized monoclonal antibody which solid-phase-ized the monoclonal antibody according to claim 1, 2, or 3 to support.
[Claim 5] The solid phase-ized monoclonal antibody according to claim 4 chosen from the group which this support becomes from the granular object, the spherical object, the tube, plate, and magnetic particle made of glass or synthetic resin.
[Claim 6] The solid phase-ized monoclonal antibody according to claim 5 this whose magnetic particle is a magnetic bead.
[Claim 7] The hybridoma which can produce a monoclonal antibody according to claim 1, 2, or 3.
[Claim 8] The hybridoma according to claim 7 chosen from the group which consists of hybridoma BA101 Shionogi (FERM BP-4004), BA20 Shionogi (FERM BP-4005), BA135 Shionogi (FERM BP-4006), and BA312 Shionogi (FERM BP-4007).
[Claim 9] The separation approach of basophilic leucocyte which a solid phase-ized monoclonal antibody and body fluid according to claim 4, 5, or 6 are made to react, and is characterized by making it combine with this solid phase-ized monoclonal antibody, and catching the basophilic leucocyte in this body fluid.
[Claim 10] The separation approach according to claim 9 chosen from the group which this body fluid becomes from blood, the pituita, tear fluid, and saliva.
[Claim 11] The isolation approach of the chemical mediator from this basophilic leucocyte characterized by making the basophilic leucocyte separated by the approach according to claim 9 or 10 react with allergen or an anti-IgE antibody.
[Claim 12] The isolation approach according to claim 11 chosen from the group which this chemical mediator becomes from a histamine, leukotriene, and a platelet activating factor (PAF).
[Claim 13] The isolation approach according to claim 11 chosen from the group which this allergen becomes from house dust, pollen, alimentary allergen, and a chemical.
[Claim 14] The chemical mediator isolation examining method from the basophilic leucocyte which has the following processes.
(1) Make a solid phase-ized monoclonal antibody and body fluid according to claim 4, 5, or 6 react. Make it combine with this solid phase-ized monoclonal antibody, and the basophilic leucocyte in this body fluid is caught. The process and (2) which separate this basophilic leucocyte Process (1)

The process which makes the separated basophilic leucocyte react with allergen or an anti-IgE antibody, and separates a chemical mediator from basophilic leucocyte, And (3) Process (2) The process, [Claim 15] which measure the amount of the chemical mediator isolated from basophilic leucocyte The isolation examining method according to claim 14 which is what is chosen from the group which this chemical mediator becomes from a histamine, leukotriene, and a platelet activating factor (PAF).

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention is (1). It reacts with basophilic leucocyte and is (2). After the formation of support solid phase holds antibody activity, and it is (3). The specific histamine isolation through IgE of this basophilic leucocyte is not checked, but the monoclonal antibody which does not induce nonspecific histamine isolation of (4) this basophilic leucocyte further, and this monoclonal antibody offer the solid phase-ized monoclonal antibody solid-phase-ized to support. Furthermore, this invention offers the separation approach of the basophilic leucocyte from the body fluid using the hybridoma and this monoclonal antibody which produce this monoclonal antibody, the isolation approach of the chemical mediator from the basophilic leucocyte separated by this separation approach, and the isolation examining method of the chemical mediator from basophilic leucocyte.
[0002]
[Description of the Prior Art] The basophilic leucocyte which is a kind of a leucocyte is the target cell of IgE, stores various chemical transmitters (chemical mediator) including a histamine into the granulation, and attracts attention as central existence of an allergic response with the mast cell.
[0003] Usually, the release mechanism of the chemical mediator from basophilic leucocyte is roughly divided into two. A bridge is constructed over the IgE comrade who combined with the receptor on the front face of the film of basophilic leucocyte by allergen and the anti-IgE antibody, degranulation starts one of them by the stimulus, it says that a chemical mediator separates, and isolation by the allergic response is equivalent to this (it is hereafter described as the specific chemical mediator isolation through IgE). Although the release mechanism or meaning are not clarified, another is the isolation produced without constructing a bridge in IgE of a direct basophilic leucocyte film front face, and happens also under an anti-IgE antibody and the nonexistence of allergen in the light of the specific isolation reaction through corresponding IgE (it is hereafter described as nonspecific chemical mediator isolation).
[0004] It is useful to examine the specific chemical mediator isolation which IgE, and a histamin release test occurs as the typical trial. [of the allergosis] [a diagnosis or] [symptoms] Especially, it is the important chemical mediator in which a histamine triggers an I-beam allergic response, and causing many reactions, such as contraction of a bronchial tube smooth muscle and sthenia of blood vessel permeability, is known. By making the gamma E-globulin (IgE) combined with the basophilic leucocyte front face in Homo sapiens peripheral blood through the receptor react with various allergen, a histamin release test is the characteristic detection method using the biological reaction which measures the amount of histamines which separated in connection with this, and, unlike a unique IgE trial (RAST), a skin test, etc. which are other allergen methods of identification, is more useful than that of cause allergen retrieval of an allergic subject as an approach near a clinical manifestation.
[0005] There are an approach of using whole blood, and an approach using a washing leucocyte in the histamin release test using this peripheral blood. Although it is thought that a whole blood method is useful when grasping a patient's allergic state synthetically, other blood serum components other than basophilic leucocyte may affect measurement of histamine isolation. For this reason, when studying the action mechanism of drugs etc. or doing fundamental research of a histamine isolation device, in analyzing the reactivity of exact basophilic leucocyte, it usually uses a washing leucocyte. However, in order to separate a washing leucocyte, after a dextran solution removes an erythrocyte, washing accompanied by centrifugal [2 - 3 times of] is performed, adjusting a white blood cell count further etc. needs to be operated complicated, for this reason, blood volume required for inspection also increases, and there are many difficulties in using as routine laboratory tests.
[0006] Moreover, in the matter emitted, existence of the chemical mediator which shows powerful blood-vessel-permeability sthenia operation and leukocyte migration ability, such as leukotriene and a platelet activating factor (PAF), is known with the degranulation of basophilic leucocyte, and the basophilic leucocyte origin chemical mediator of these versatility is becoming the quality of a fundamental-research object for the purpose of the symptoms analysis of allergy. However, these chemical mediators have many which will be metabolized promptly,

when emitted into blood, and especially in case they perform these measurement, they serve as requirements with important handling [a sample] quickly and strictly. Current analysis is very difficult for many of such basophilic leucocyte origin chemical mediators.
[0007]
[Problem(s) to be Solved by the Invention] As mentioned above, there are specific isolation which minded [of the chemical mediator from basophilic leucocyte] IgE by the allergic response, and the other nonspecific isolation, and it is required for a diagnosis of the allergosis to measure the chemical mediator which separated specifically through IgE. However, in order to perform a histamin release test, without being influenced of a blood serum component as mentioned above and to have to separate a leucocyte, complicated actuation and a lot of blood are required, and it is unsuitable for everyday inspection. Furthermore, since the handling of a sample is complicated also about chemical mediators other than a histamine, a difficult situation performs the present analysis.
[0008] Then, when this invention persons separated basophilic leucocyte out of blood and having been used for the isolation trial of a histamine etc., they inquired by thinking that the above-mentioned problem will be solvable. As separation and a purification method of basophilic leucocyte until now By removing an impurity cell from the sample which raised the consistency of basophilic leucocyte beforehand using the magnetic particle which combined the antibody reacted to cell components other than basophilic leucocyte by the specific gravity centrifuge method P.J. Frederick's and others (P.J.Frederik) method of refining basophilic leucocyte [Journal OBU immuno logical MESOZZU (J.Immunol.Methods) 149 and 207] (1992) etc. -- it is. However, since this approach is not the purification method that separates the basophilic leucocyte itself made into the purpose but a purification method by removing impurity, effectiveness of operability is complicated [an approach] to a bad top.
[0009] on the other hand, many antibodies reacted to the basophilic leucocyte itself are also got to know -- having -- **** -- [-- for example P. -- BARENTO et al. (Valent) and International AKAIBUZU allergy applied immunology (Int.Arch.Allergy Appl.Immunol.) -- 91 and 198 (1990)] Moreover, the monoclonal antibody which shows a specific reaction to Homo sapiens basophilic leucocyte is also reported. [M. P. BOJA et al. (Bodger), brad (Blood) 69 and 1414] (1987) . However, the example which used for the chemical mediator isolation trial of a histamine etc. the Homo sapiens basophilic leucocyte which this basophilic leucocyte reactivity antibody was combined with solid phase support, and the example of a success of the attempt used for separation of Homo sapiens basophilic leucocyte was not known, therefore was separated by such [naturally] approach is not seen, either.
[0010] this fact also considers failure by the loss of basophilic leucocyte reactivity by combining a basophilic leucocyte reactivity antibody with solid phase support etc. -- having (example 1 of the below-mentioned experiment (1)) -- the biggest cause the case where the specific histamine isolation which the basophilic leucocyte isolated preparatively using this basophilic leucocyte reactivity antibody has received the damage, and minded IgE is checked -- [-- for example P. J. Frederick et al., journal OBU immuno logical MESOZZU (J. Immunol.Methods) 149, and 213 (1992), and J.T. Schrader (J.T.Schroeder) ** -- Since nonspecific histamine isolation became remarkably high as compared with the specific histamine isolation through journal OBU immuno logical MESOZZU (J.Immunol.Methods) 133, 269 - 277 (1990)], or IgE, It can consider that it is impossible to be equal to use of the quantum of a histamine isolation reaction etc. in many cases (example 1 of the below-mentioned experiment (2)). P.J. glimpse that Frederick and others refines basophilic leucocyte using cell components other than basophilic leucocyte, and the antibody which reacts -- it is understood as it having been for having taken the roundabout approach also avoiding an above-mentioned fault.
[0011]
[Means for Solving the Problem] However, in the basophilic leucocyte itself, if separation purification of basophilic leucocyte can be performed using a taste base ball reactivity antibody as a target, achievement of simple and highly precise purification should be attained. Then, this invention persons are monoclonal antibodies, the antibody, i.e., the basophilic leucocyte, which can be used for this purpose, which react, and searched for the monoclonal antibody which has the property of having the reactivity over basophilic leucocyte even after combining with solid phase support, and after association not giving a damage to the basophilic leucocyte concerned with basophilic leucocyte, namely, not having significant effect on the specific histamine isolation and nonspecific histamine isolation through IgE wholeheartedly. Consequently, it succeeded in preparation of the target monoclonal antibody. And after having solid-phase-ized this antibody to support, making it react with body fluid and catching the basophilic leucocyte in this body fluid by this monoclonal antibody, it succeeded in establishing the approach of separating basophilic leucocyte easily, making allergen or an anti-IgE antibody react to the separated basophilic leucocyte, and separating a histamine by removing unreacted body fluid. Furthermore, this invention persons establish the specific histamin release test method through IgE of the basophilic leucocyte separated using this antibody, and came to complete this invention.
[0012] That is, this invention offers the monoclonal antibody which has the following properties.
(1) Basophilic leucocyte and (2) which react (3) to which after the formation of support solid phase holds antibody activity (4) which does not check the specific histamine isolation through IgE of this basophilic leucocyte [0013]

which does not induce nonspecific histamine isolation of this basophilic leucocyte The above which this invention offers (1) (2) (3) And (4) It sets to the monoclonal antibody which has a property, and is (1). It means that this monoclonal antibody reacts reacting with basophilic leucocyte with the surface antigen of basophilic leucocyte.
[0014] Moreover, (2) Even after combining [support solid phase-ization and] this monoclonal antibody with support as holding antibody activity, it means not losing prehension ability of basophilic leucocyte. As this support, although all can be used if a magnetic particle like glass, the granular object made of synthetic resin (bead) or a spherical object (ball), a tube, a plate, and a magnetic bead etc. is the support used when usually solid-phase-izing an antibody for example, it is good to use a magnetic particle preferably.
[0015] The basophilic leucocyte prehension ability of a solid phase-ized antibody is judged by authorizing whether the solid-phase-ized monoclonal antibody combined with basophilic leucocyte, specifically makes a histamine an index, and performs it by the following approaches. First, the solid-phase-ized Homo sapiens basophilic leucocyte and the monoclonal antibody which reacts are made to react to a magnetic bead with Homo sapiens blood. subsequently, the buffer solution for the histamine isolation after collecting beads magnetically and removing supernatant liquid (a calcium chloride --) Add the HEPES buffer solution containing a magnesium chloride etc., and freeze thawing is repeated several times. Histamine gaging system according the total amount of histamines of the supernatant liquid to the known approach, for example, HPLC method, [Y. TSURUTA et al. (Tsuruta), a journal OBU chromatography (J.Chromatogr.) -- 224 and 105 (1981)] (Shimadzu) It measures and the antibody by which the histamine was detected is chosen as an antibody which has basophilic leucocyte prehension ability.
[0016] Moreover, (3) If the specific histamine isolation through IgE of this basophilic leucocyte is not checked, the basophilic leucocyte combined with this monoclonal antibody means maintaining substantially the specific histamine isolation ability through IgE which it originally has in blood. It can check whether it maintains substantially by comparative experiments with monoclonal antibody BA312 discovered in this invention. namely, from the basophilic leucocyte caught by BA312 maintaining the histamine isolation ability which it originally has in blood (example 2 of the below-mentioned experiment) A ******-ed-ized monoclonal antibody is made to react with basophilic leucocyte on solid phase-ized BA312 and these conditions. When the amount of histamines which performed anti-IgE antibody processing and separated under these conditions is 60% or more of that of BA312, the monoclonal antibody concerned judges the obtained both monoclonal antibody joint basophilic leucocyte to be what "does not check the specific histamine isolation through IgE of this basophilic leucocyte." When it comes to the object of this invention, it determines.
[0017] Furthermore, (4) The amount of the nonspecific histamine isolation from the basophilic leucocyte combined with this monoclonal antibody as not inducing nonspecific histamine isolation of this basophilic leucocyte means that it minded IgE that it is 30% or less of the amount of specific histamine isolation.
[0018] The amount of nonspecific histamine isolation can be obtained by measuring the amount of the histamine which separates by adding the hydroxyethyl piperazine-N'-2-ethane-sulfonic-acid (HEPES) buffer solution (buffer solution for histamine isolation) containing the suitable solvent for this basophilic leucocyte, for example, a calcium chloride, a magnesium chloride, a human serum albumin (HSA), etc., and making it react for 10 - 60 minutes at 10-50 degrees C, the known approach, for example, HPLC method.
[0019] The amount of specific histamine isolation can be obtained by measuring the amount of the histamine which separates by making this basophilic leucocyte and the anti-IgE antibody prepared with the buffer solution for histamine isolation through IgE react under the same temperature and reaction time as a top under the same approach and conditions as a top.
[0020] The production approach of the monoclonal antibody of this invention is shown briefly below.
(1) Carry out immunity of the preparation mouse of a hybridoma by Homo sapiens basophilic leucocyte. Immunity is performed by repeating Homo sapiens basophilic leucocyte in the abdominal cavity of a mouse, hypodermically, or a vein several times, and inoculating it into it every several weeks. An antibody forming cell is obtained from the mouse by which immunity was carried out, a myeloma cell is united with this, and a hybridoma is produced. The well of the hybridoma which produces the antibody reacted to the inner Homo sapiens basophilic leucocyte of the obtained hybridoma is chosen.
[0021] After a sheep anti-mouse immunoglobulin antibody combines the antibody to which cloning of the selected hybridoma was carried out, and the hybridoma clone which appeared produced it with the magnetic bead combined beforehand, the property about the prehension ability of the basophilic leucocyte in Homo sapiens blood and the separated histamine isolation of basophilic leucocyte is authorized. Assay of prehension ability can be performed by the above-mentioned approach.
[0022] Assay of the property about histamine isolation of the separated basophilic leucocyte This solid-phase-ized antibody is made to react to a magnetic bead with Homo sapiens blood like assay of prehension ability. After collecting beads magnetically and removing supernatant liquid, according to the above-mentioned approach, the amount of nonspecific histamine isolation and the amount of the specific histamine isolation through IgE are measured. The antibody, i.e., the antibody of the amount of the specific histamine isolation the amount of

nonspecific histamine isolation minded IgE which became 10% or less preferably 30% or less, by which the specific histamine isolation through IgE is not checked, and nonspecific histamine isolation is not promoted is chosen.
[0023] Thus, the hybridoma clone which produces the antibody which does not check the specific histamine isolation through IgE of the basophilic leucocyte which does not lose antibody activity, but has the prehension ability of basophilic leucocyte even after combining with support, and was separated, and does not induce nonspecific histamine isolation is chosen, this is cultivated, and monoclonal antibodies are collected.
[0024] Selection of the above-mentioned immunization, a cell fusion method, and syncytium, cloning, etc. can be performed by the well-known usual approach.
[0025] (2) It is in vitro (inch vitro) cultivation or in vivo (invivo) about the hybridoma in which the monoclonal antibody made production selection. It is made to increase by cultivation and a monoclonal antibody is obtained. As in vitro cultivation, a hybridoma can be cultivated until it becomes a growth limitation by the RPMI culture medium (perfect RPMI culture medium) containing fetal calf serum, and a monoclonal antibody can be obtained by collecting the culture supernatants. A hybridoma is transplanted to the abdominal cavity of the mouse which carried out intraperitoneal administration processing of PURISUTEN beforehand as in vivo cultivation, several weeks after, abdomen hypertrophy of a mouse can be confirmed, the ascites can be extracted, and a monoclonal antibody can be obtained for IgG or an IgM fraction from ascites separation and by refining, combining suitably known approaches, such as ammonium sulfate fractionation and a DEAE sepharose column.
[0026] The hybridoma which produces the monoclonal antibodies BA101, BA20, BA135, and BA312 of this invention obtained by the above-mentioned approach From September 10, 1992, to the Fermentation Research Institute in Japan 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken (zip code 305), the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry Respectively "BA101 Shionogi" (Fermentation Research Institute ****** No. 4004, FERM BP-4004), "BA20 Shionogi" (Fermentation Research Institute ****** No. 4005, FERM BP-4005), Based on Budapest Treaty, it ****s as "BA135 Shionogi" (Fermentation Research Institute ****** No. 4006, FERM BP-4006) and "BA312 Shionogi" (Fermentation Research Institute ****** No. 4007, FERM BP-4007). Thus, the monoclonal antibody of this invention was mentioned above although what is produced by the hybridoma deposited, for example was mentioned. (1) - (4) If it is the monoclonal antibody which has the property, all are the range of this invention. Monoclonal antibody BA312 is used preferably.
[0027] The monoclonal antibody of this invention reacts with the leucocyte containing Homo sapiens basophilic leucocyte, and a human erythrocyte does not react. Furthermore, even after solid-phase-izing to support, the monoclonal antibody of this invention can fully catch basophilic leucocyte, does not induce isolation of the nonspecific chemical mediator of the caught basophilic leucocyte, and does not check the specific chemical mediator isolation through IgE. Moreover, the monoclonal antibody of this invention can be advantageously used as a solid phase-ized monoclonal antibody which is combined with the above support and obtained. It is not limited and especially the method of making it combine with support and making a monoclonal antibody solid-phase-ize is a well-known approach et al., for example, [T. Lee, (Lea) and SUKANJINABIAN journal OBU immunology (Scand.J.Immunol.). 22 and 207 (1985)] can perform. For example, 0-50 degrees C of monoclonal antibodies are made to react preferably at 4-40 degrees C for 30 minutes to 48 hours for 5 minutes to 72 hours with the solid phase support by which the coat was carried out by ANKOTEDDO, the compound with a chemical active group like a tosyl group, or the anti-mouse immunoglobulin.
[0028] This invention is the above and offers the separation approach of basophilic leucocyte which the solid phase-ized monoclonal antibody and body fluid of this invention which are made and obtained are made to react, and is characterized by making it combine with this solid phase-ized monoclonal antibody, and catching the basophilic leucocyte in this body fluid again.
[0029] As this body fluid, although blood, the pituita, tear fluid, saliva, etc. are mentioned, blood is used preferably.

[0030] By the way, G. GODA naks report the approach of combining an antibody with a magnetic particle and separating the target cell. [G. GODA nak et al. (Gaudernack), journal OBU immuno logical method (J.Immunol.Methods) 90, 179] (1986), etc. are known widely. Moreover, the method of refining basophilic leucocyte by removing an impurity cell from the sample which raised the consistency of basophilic leucocyte beforehand using the magnetic particle which combined the antibody reacted to cell components other than basophilic leucocyte by the specific gravity centrifuge method P. It is reported by J. Frederick and others. [P. J. Frederick et al. (Frederic), Journal OBU immuno logical method (J.Immunol.Methods) 149 and 207 (1992)] With this invention There is an essential difference in the part whether to use an antibody [as opposed to cells other than basophilic leucocyte for whether the antibody to basophilic leucocyte is used].
[0031] On the other hand, the separation approach of the basophilic leucocyte of this invention the monoclonal antibody of this invention Support, For example, to a magnetic particle, solid-phase-ize through a direct or anti-mouse immunoglobulin, and it considers as an antibody joint magnetic particle. Preparation separation of the cell

population which contains basophilic leucocyte by high concentration by simple actuation of drawing a magnetic particle near for this using a magnet after making 0-50 degrees C carrying out a short-time (1 - 60 minutes) reaction at 15-40 degrees C preferably with blood, body fluid and, and removing an unreacted sample can be carried out.
[0032] This invention offers the isolation approach of the chemical mediator from this basophilic leucocyte characterized by making the basophilic leucocyte separated with the separation method of the basophilic leucocyte of this invention react with allergen or an anti-IgE antibody again.
[0033] In addition to this as allergen, a chemical etc. can use suitably the things usually used by allergy diagnosis including alimentary allergen, such as inhalant allergen, such as house dust and pollen, meat, and an egg, if needed.
[0034] 10-50 degrees C of reactions with this basophilic leucocyte, allergen, or an anti-IgE antibody are preferably performed for 10 - 60 minutes for 0.5 - 300 minutes at 25-40 degrees C.
[0035] This invention is (1) of further the following. - (3) The chemical mediator isolation examining method from basophilic leucocyte which consists of a process is offered.
(1) The process, and (2) which the solid-phase-ized monoclonal antibody and the body fluid of this invention are made to react, and are made to combine the basophilic leucocyte in this body fluid with this solid-phase-ized monoclonal antibody, catch, and separate this basophilic leucocyte Process (1) The process which makes the separated basophilic leucocyte react with allergen or an anti-IgE antibody, and separates a chemical mediator from basophilic leucocyte, and (3) Process (2) The process, [0036] which measure the amount of the chemical mediator isolated from basophilic leucocyte Above (1) And (2) It is good to carry out according to the separation approach of the basophilic leucocyte of aforementioned this invention, and the isolation approach of the chemical mediator from basophilic leucocyte, respectively.
[0037] Above (3) What is necessary is just to measure measurement of the amount of the chemical mediator which separated by the known approach suitably according to the chemical mediator made into the purpose. For example, in the case of a histamine, it is a histamine gaging system by the HPLC method. [Y. TSURUTA et al. (Tsuruta), Journal OBU chromatography (J.Chromatogr.) By 224 and 105(1981)] (Shimadzu), in leukotriene and PAF ******** [E. Hayes et al. (Hayes) and journal OBU immunology (J.Immunol. 131 and 429 (1983) --) And it can measure by [M.A. SUMARU et al. (Smal), journal OBU immuno logical MESOZZU (J.Immunol.Methods) 128, and 183 (1990)]. That is, measurement of the histamine by HPLC is performed as follows, for example. Through a sample, an orthochromatic phthalaldehyde and NaOH are added 0.1% at the histamine which carried out separation elution, and it reacts to the column filled up with cation exchange resin at 45 degrees C, and let a histamine be a fluorescent substance. Next, it is H2 SO4. Fluorescence intensity is measured, after making it react and performing stabilization of a fluorescent substance, and enhancement of fluorescence intensity.
[0038] Moreover, after measurement of the leukotriene by RIA makes a sample or standard non-indicator leukotriene, an anti-leukotriene antibody, and 4 degrees C of 3 H indicator leukotrienes react for 5 to 24 hours, it is made to react for 10 minutes among a dextran coated charcoal and ice. Supernatant liquid is isolated preparatively after centrifugal and radioactivity is measured. Moreover, the measurement of PAF by RIA measures the radioactivity of **** obtained by centrifugal separation, after making a sample or the standard non-indicator PAF, an anti-PAF antibody, and 125 I indicators PAF and a second antibody react at a room temperature for 5 to 24 hours. Moreover, it can measure by RIA also about the measuring method of a histamine. When measuring by RIA, a sample or a standard non-indicator histamine, an anti-histamine antibody, and 0-40 degrees C of 125 I-indicator histamines are made to react preferably at 0-10 degrees C for 30 minutes to 24 hours for 5 minutes to 48 hours. A B/F separating medium, for example, a polyethylene glycol, is added after that, a reaction is performed for 10 minutes in ice, and the radioactivity of **** obtained by centrifugal is measured.
[0039] Furthermore, EIA can also perform as a measuring method of a histamine. That is, all well-known enzyme immunoassay, such as a sandwich technique, the competing method, and ELISA, is approaches (Luminescence Immunology and Molecular Application, CRC Press pp 2-10, 1990) preferably reported by KUNOKKUSU van motorbike (Knox Van Dyke) although it can use. It follows and measures. That is, the mixture of a sample, the standard histamine which carried out the biotin indicator, and an enzyme-labeling anti-histamine antibody is made to react with the support which solid-phase-ized avidin. The enzyme activity of the biotin indicator histamine-enzyme-labeling anti-histamine antibody complex combined with avidin solid phase-ized support after washing is measured. All the things for which support and an enzyme are usually used with enzyme immunoassay can be used. As support, horseradish peroxidase (HRP) is preferably used as a microplate and an enzyme.
[0040] In order to remove the effect of the nonspecific chemical mediator isolation from the separated basophilic leucocyte in the method of examining this invention, according to the above-mentioned approach, the total amount of chemical mediators (it applies to the measuring method of the total amount of histamines correspondingly) and the amount of nonspecific chemical mediator isolation are also measured to coincidence, and it is following formula: [0041].
[Equation 1]

特異的ケミカルメディエーター遊離率（%）＝

$$\frac{\text{IgE 特異的遊離ケミカルメディエーター量} - \text{非特異的遊離ケミカルメディエーター量}}{\text{総ケミカルメディエーター量} - \text{非特異的遊離ケミカルメディエーター量}} \times 100$$

[0042] It is desirable to compute the specific rate of chemical mediator isolation which was alike and minded IgE more.

[0043]

[Example] Hereafter, although an example and the example of an experiment explain this invention in more detail, this invention is not limited at all by these examples etc.

1. Production of monoclonal antibody reacted to basophilic leucocyte (1) To two female BALB/c mice of 8 weeks old of immunity, it is 1x106, respectively. The Homo sapiens basophilic leucocyte of an individual is injected intraperitoneally and it is 1x106 3 times per three weeks henceforth, respectively. The Homo sapiens basophilic leucocyte of an individual was injected intraperitoneally.

[0044] (2) Three days after the cell fusion last immunity, the spleen was extracted from two mice and cell suspension was prepared using the RPMI culture medium. This 2x108 The splenic cells of an individual, and 6x107 Myeloma cell NS-1 of an individual was mixed, and in addition, it agitated for 1 more minute after centrifugation, agitating gently polyethylene-glycol (average molecular weight 4000) 1ml 50% to precipitate. For 1 minute was required for 1ml of RPMI culture media, in addition, after adding 1ml similarly, further, for 3 minutes was required and 7ml was added. precipitate is floated after centrifugation to 40ml (perfect RPMI culture medium) of RPMI culture media which contain fetal calf serum 15% -- making -- four 96 hole micro culture plates -- each -- it inoculated 0.1ml into the well at a time, and cultivated at 37 degrees C under existence of 7% carbon dioxide gas. The 0.1 ml perfect RPMI culture medium (HAT medium) containing hypoxanthine 100microM, aminopterin 0.4microM, and thymidine 16microM was added 24 hours after. 2, 3, 5, and eight days [culture initiation and] after, the 0.1 ml culture supernatant was thrown away, and the 0.1 ml HAT medium was added. The 0.1 ml culture supernatant was thrown away on 11 and the 14th, and the 0.1 ml perfect RPMI culture medium (HT culture medium) containing hypoxanthine 100microM and thymidine 16microM was added. The colony of a hybridoma appeared from all wells of a total of 384.

[0045] (3) the selective culture of a hybridoma -- a well -- the inner hybridoma was cultivated by the perfect RPMI culture medium, and the existence of the specific antibody production in the culture supernatant was detected as follows.

[0046] After the dextran physiology salt solution settled 5ml, in addition an erythrocyte to 20ml of Homo sapiens peripheral blood 5%, the leucocyte of supernatant liquid was divided into it. The cell which consists of specific gravity 1.07 to 1.08 was separated from this leucocyte by the discontinuity specific gravity centrifuge method using PARCOR prepared to specific gravity 1.07 and 1.08. About a separation cell, it is the Alcian-blue method. [S.G. Harriet (Harriet) et al., Brad (Blood) 46, 279 (1975)] It dyes, the number of basophilic leucocytes is calculated, and it is 4x105 as basophilic leucocyte. So that it may be set to an individual/ml It prepared with the 10mM hydroxyethyl piperazine-N'-2-ethane-sulfonic-acid (HEPES) buffer solution (pH 7.4) (HA-HEPES buffer solution) containing a 0.8% sodium chloride, 0.037% potassium chloride, and a 0.03% human serum albumin (HSA). To a test tube, it is 2x104 as the number of basophilic leucocytes. The separation cell was poured distributively so that it might become an individual. The culture supernatant of a hybridoma was 50microl Added to this, and it was made to react at 37 degrees C for 1 hour. 50micro of mixed liquor l of the full ORESSEN isothiocyanate (FITC) indicator goat anti-Homo sapiens IgE antibody after the HA-HEPES buffer solution washes [1-time], and a phycoerythrin indicator goat anti-mouse immunoglobulin antibody -- in addition, it was made to react at a room temperature for 1 hour It judged whether the HA-HEPES buffer solution would perform observation by the fluorescence microscope after 1-time washing, and the indicator of the cell by which the FITC indicator was carried out would be carried out by phycoerythrin. Thus, that to which the monoclonal antibody contained in the culture supernatant of a hybridoma reacts was chosen 100 well to the basophilic leucocyte by which the FITC indicator was carried out.

[0047] After carrying out cloning of the hybridoma chosen like the above by limiting dilution, it cultivated to the growth limitation by flask culture, respectively. It was made to react at 4 degrees C for 16 hours in addition to magnetic bead 15mg with which the sheep anti-mouse immunoglobulin antibody has combined 1ml of this culture supernatant beforehand, performing loose churning, and the monoclonal antibody solid phase-ized magnetic bead was obtained. these monoclonal antibody solid phase-ized magnetic beads -- using -- an approach given in the following example 4 -- following -- the basophilic leucocyte prehension ability from Homo sapiens peripheral blood, and IgE of the separated basophilic leucocyte -- the property about specific histamine isolation and nonspecific histamine isolation was investigated. Consequently, it has the aforementioned property, and hybridoma BA101 Shionogi (FERM BP-4004) which produces the monoclonal antibodies BA101, BA20, BA135, and BA312

suitable for separation of the cell population containing the Homo sapiens basophilic leucocyte of this invention and the histamine isolation test method of a separation cell, BA20 Shionogi (FERM BP-4005), BA135 Shionogi (FERM BP-4006), and BA312 Shionogi (FERM BP-4007) were chosen so that clearly from the example of the after-mentioned experiment.

[0048] Moreover, as a result of investigating the class of the mouse immunoglobulin to which the four above-mentioned sorts of monoclonal antibodies belong, BA20, BA101, and BA135 belonged to IgG1, and it became clear that BA312 belonged to IgM.

[0049] (4) Production of a monoclonal antibody (a) in vitro The cultivation hybridoma was cultivated until it became a growth limitation (1x106 an individual/ml) by the perfect RPMI culture medium, and the culture supernatants were collected.

[0050] (b) in vivo To the BALB/c mouse which carried out intraperitoneal administration processing by 0.5 ml ******** PURISUTEN, it is 5x106. The hybridoma of an individual was transplanted to intraperitoneal. Abdomen hypertrophy of a mouse was confirmed after about three weeks, and the ascites was extracted.

[0051] (5) Purification of a monoclonal antibody (a) The ascites obtained at the protein A affinity chromatography method front process was salted out with the sodium-sulfate solution 18%, and the produced precipitation was dialyzed with this liquid after dissolving in a 0.01M boric-acid buffer saline solution (pH 8.0). 20mg of monoclonal antibodies obtained by the salting-out was melted to 2ml (pH 8.0) of 0.01M boric-acid buffer saline solution, and it was made to stick to the column (1.6x5cm) of protein A-sepharose [Pharmacia Corp. (Pharmacia AB)]. First, the impurity was flowed out by about 50ml (pH 8.0) of 0.01M boric-acid buffer saline solution, subsequently it was eluted by about 100ml (pH 4.0) of 0.01M citric-acid buffer saline solution, and the purification monoclonal antibodies BA20, BA101, and BA135 were obtained.

[0052] (b) 20mg of monoclonal antibodies obtained by the sepharose CL-4B gel chromatography method aforementioned salting-out was melted to 2ml of 0.01M boric-acid buffer saline solution, fractionation was let pass and carried out to the column (1.6x70cm) of sepharose CL-4B [Pharmacia Corp. (PharmaciaAB)], the Maine peak was isolated preparatively, and purification monoclonal antibody BA312 was obtained.

[0053] 2. Solid phase-ized DINA bead to secondary antibody joint bead of monoclonal antibody M-450 The 30 mg-beads/ml suspension of a sheep anti-mouse immunoglobulin [dynal company (Dynal)] was isolated preparatively in 1ml test tube after churning. After collecting beads magnetically and removing supernatant liquid, 1ml of hybridoma culture supernatants was added, and the reaction was performed at 4 degrees C for 16 hours, performing loose churning. the HA-HEPES buffer solution after collecting beads magnetically and removing supernatant liquid -- 4 times and 4 degrees C -- every [a for / 30 minutes] -- it washed performing loose churning and the monoclonal antibody solid phase-ized magnetic bead was obtained. Moreover, 1ml (20microg/(ml)) of purification monoclonal antibodies prepared with the HA-HEPES buffer solution was added instead of the hybridoma culture supernatant, and the monoclonal antibody solid phase-ized magnetic bead was obtained by the same approach as the above.

[0054] 3. Solid phase-ized DINA bead ANKOTEDDO [to ANKOTEDDO bead of monoclonal antibody M-450 (the 30 mg-beads/ml suspension of dynal company (Dynal)] was isolated preparatively in 1ml test tube after churning.) After collecting beads magnetically and removing supernatant liquid, 1ml (0.3 mg/ml) of 0.05M tris-hydrochloric-acid buffer-solution (pH9.5) solutions of BA312 was added, and it agitated well. The reaction was performed at 4 degrees C for 16 hours, performing loose churning. 0.01M which contain 0.5%HSA after collecting beads magnetically and removing supernatant liquid The HEPES buffer solution (pH 7.4) performed washing every 4 times during 5 minutes, the HA-HEPES buffer solution performed washing at 4 degrees C further for 16 hours, and BA312 solid-phase-ized magnetic bead was obtained.

[0055] 4. 40ml of HA-HEPES buffer solutions was added to the cellular segregation by the monoclonal antibody solid phase-ized magnetic bead, and 10ml of healthy human blood liquid which collected blood in histamine isolation reaction EDTA blood collecting tubing, and blood was prepared 20%. The magnetic bead which solid-phase-ized the culture supernatant of hybridoma BA20 Shionogi, BA101 Shionogi, BA135 Shionogi, and BA312 Shionogi, respectively was prepared so that it might be set to 3 mg-beads / ml with the HA-HEPES buffer solution. 20% blood -- a test tube -- every [500microl] -- pouring distributively -- here -- the above BA20, BA101, and BA135 and the formation of BA312 solid phase -- magnetic bead 50microl was added and the reaction was performed for 5 minutes under the loose shaking condition at the room temperature. After collecting beads magnetically and removing supernatant liquid, 500micro of HA-HEPES buffer solutions l performed washing once. After washing termination, a 0.5mM calcium chloride, a 0.25mM magnesium chloride, A 0.1% glucose, a 0.9% sodium chloride, 0.035% sodium bicarbonate, And 20mM(s) containing 0.1%HSA HEPES buffer solution (pH 7.0) (buffer solution for histamine isolation), Or it agitated by adding 500micro (HE-69B) of monoclonal anti-Homo sapiens IgE antibodies l prepared so that it might become in ml and 4microg /with the buffer solution for histamine isolation. The total amount of histamines of a separation cell made the specimen what repeated freeze thawing 3 times and performed it by the specimen which added the buffer solution for histamine isolation. IgE -- the specific histamine isolation reaction was performed by making the specimen which added the monoclonal anti-Homo

sapiens IgE antibody react at 37 degrees C for 1 hour. Moreover, the nonspecific histamine isolation reaction was performed by making the specimen which added the buffer solution for histamine isolation react at 37 degrees C for 1 hour. It agitated after reaction termination, and after carrying out at-long-intervals ** for 1500rpmx 5 minutes, 300micro of supernatant liquid l was isolated preparatively. Measurement of the amount of isolation histamines is a histamine gaging system by the HPLC method. [Y. TSURUTA (Tsuruta) et al., journal OBU chromatography (J.Chromatogr.) 224 and 105] (1981) (Shimadzu) performed.

[0056] As shown in Table 1, when the total amount of histamines of the cell separated with BA20, BA101, BA135, and BA312 solid-phase-ized magnetic bead was measured, it turned out that a histamine is detected in all antibodies and it has the separability of basophilic leucocyte. moreover, it is shown in drawing 1 -- as -- IgE of a separation cell -- the specific rate of histamine isolation -- good -- in addition -- and nonspecific histamine isolation was suppressed to 30% or less as compared with specific histamine isolation.

[0057]

[Table 1]

	BA20	BA101	BA135	BA312	非固相ビーズ
血液検体	++	++	++	++	-

好塩基球の分離能　++ : 有り　- : なし

[0058] Comparison with an example of experiment 1.CD antibody, and this invention monoclonal antibody (1) Basophilic leucocyte prehension ability P. BARENTO's and others report [P. BARENTO et al. (Valent), International archives allergy applied immunology (Int.Arch.Allergy Appl.Immunol.) It is based on 91 and 198 (1990)]. CD antibody reacted to the antigen well discovered on a basophilic leucocyte front face, i.e., CD9 antibody, (TP82 and NICHIREI CORP.) The antibody solid phase-ized magnetic bead was prepared by the approach shown in 2 of the above-mentioned example using each antibody of a CD11b antibody (BEAR1, immuno tech company), CD13 antibody (MCS2 and NICHIREI CORP.), and CD32 antibody (two E1, immuno tech company). The specific cell population was separated by the same approach as 4 of an example using above-mentioned CD antibody and BA20, and BA312 solid-phase-ized magnetic bead, and the total amount of histamines of the separation cell origin was measured.

[0059] Although good basophilic leucocyte prehension was accepted in CD9 antibody, a CD11b antibody, and BA20 and BA312 as shown in Table 2, separation of basophilic leucocyte was not accepted in CD13 antibody and CD32 antibody.

[0060]

[Table 2]

	CD9	CD11b	CD13	CD32	BA20	BA312
血液検体A	++	++	-	-	++	++
血液検体B	++	++	-	-	++	++

好塩基球捕捉能　++ : 有り　- : なし

[0061] (2) 1. (1) of the example of the histamine isolation ability above-mentioned experiment of a separation cell Inside of examined CD antibody, CD9 antibody basophilic leucocyte prehension ability was accepted to be, a CD11b antibody, and the histamine isolation ability of the separation cell by each antibody solid phase-ized magnetic bead of BA20 and BA312, That is, three items of the total amount of histamines, the amount of IgE specific isolation histamines, and the amount of nonspecific isolation histamines were measured by the same approach as 4 of the above-mentioned example using the healthy human blood liquid of 20 examples.

[0062] consequently -- while checking separation of basophilic leucocyte by all antibodies -- the difference for every blood specimen -- all the antibodies of a certain thing -- IgE -- specific histamine isolation was accepted.

[0063] In this invention, if the ratio of the amount of the nonspecific histamine isolation through IgE of the basophilic leucocyte caught and separated by the antibody solid-phase-ized in the MAGUNETCHIKKU bead to the amount of specific histamine isolation is 30% or less, it will be thought that this antibody is useful by the chemical mediator isolation examining method, and it will be especially thought at 10% or less that it is useful. CD9 antibody and a CD11b antibody -- IgE -- since the specimens which show remarkable high nonspecific histamine isolation have occurred frequently as compared with specific histamine isolation, it is shown in Table 3 -- as -- the above-

mentioned ratio -- 30% -- large -- exceeding -- **** -- IgE from these separation cells -- it was very difficult to perform analysis about specific histamine isolation. On the other hand, BA312 which is the monoclonal antibody of this invention was extremely excellent in this ratio with 7.5%.

[0064]

[Table 3]

モノクローナル抗体	CD9	CD11b	BA20	BA312
Non-Spec./Spec.(%)	46.9	39.7	22.0	7.5
SD	17.6	28.5	15.1	5.3

Non-Spec. ／Spec. ：ＩｇＥを介した特異的なヒスタミン遊離の量に対する非特異的なヒスタミン遊離の量の比率

SD：標準偏差

[0065] as mentioned above, the rate of basophilic leucocyte prehension -- high -- IgE -- specific histamine isolation -- good -- in addition -- and it was a separation cell by BA20 and BA312 solid-phase-ized magnetic bead that nonspecific histamine isolation is suppressed most. The separation cell by BA312 solid-phase-ized magnetic bead was especially most suitable for performing separation of the basophilic leucocyte from the Homo sapiens blood of this invention, and the histamin release test of this separation cell.

[0066] 2. Comparison examination of the histamin release test by whole blood and the separation cell was performed using whole blood and one histamin release test healthy people volunteer blood by the separation cell. By the approach by whole blood, it collected blood in 10ml of blood in heparin blood collecting tubing first. every [100micro of this blood l] was poured distributively in the test tube, and it stirred by adding the monoclonal anti-Homo sapiens IgE antibody (HE-69B) prepared so that it might be set to 50000, 5000, 500, 50, and 5 ng(s)/ml at this with the buffer solution for histamine isolation, or the buffer solution for histamine isolation 400microl every, respectively (a monoclonal anti-Homo sapiens IgE antibody is set to final concentration 40000, 4000, 400, and 40 and 4 ng(s)/ml). The total amount of histamines made the specimen what repeated freeze thawing 3 times and performed it by the specimen which added the buffer solution for histamine isolation. IgE -- the specific histamine isolation reaction was performed by making the specimen which added the monoclonal anti-Homo sapiens IgE antibody react at 37 degrees C for 1 hour. Moreover, the nonspecific histamine isolation reaction was performed by making the specimen which added the buffer solution for histamine isolation react at 37 degrees C for 1 hour. It stirred after reaction termination, and after carrying out at-long-intervals ** for 1500rpmx 5 minutes, 300micro of supernatant liquid l was isolated preparatively. The histamine gaging system (Shimadzu) by the HPLC method performed measurement of the amount of isolation histamines.

[0067] By the approach by the separation cell, cellular segregation and the histamin release test of a separation cell were performed by the approach of 4 of the above-mentioned example, and the same approach. The monoclonal anti-Homo sapiens IgE antibody (HE-69B) was prepared so that it might be set to 40000, 4000, 400, 40, and 4 ng (s)/ml with the buffer solution for histamine isolation, and it asked for the rate of histamine isolation by this. The rate of specific histamine isolation is computed in a degree type.

[0068]

[Equation 2]

$$\text{特異的ヒスタミン遊離率（\%）} = \frac{\text{IgE 特異的遊離ヒスタミン量} - \text{非特異的遊離ヒスタミン量}}{\text{総ヒスタミン量} - \text{非特異的遊離ヒスタミン量}} \times 100$$

[0069] As shown in drawing 2 , the rate of specific histamine isolation obtained the equivalent result by the approach by whole blood, and the approach by the separation cell. This showed that the separation cell obtained using the monoclonal antibody of this invention was maintaining the histamine isolation ability which it originally has in blood.

[0070] 3. Cellular segregation and the histamin release test of a separation cell were performed by the same approach as 4 of the above-mentioned example using BA312 solid-phase-ized magnetic bead prepared by the approach shown in 3 of the above-mentioned example about two child atopic dermatitis patient blood specimens whose histamin release test albumens of the separation cell by the clinical specimen are cause allergen. The albumen allergen used for the trial was prepared so that it might be set to 4000, 400, 40, 4, and 0.4 ng/ml with the buffer solution for histamine isolation, and it computed the rate of IgE specific histamine isolation by this according

to the above-mentioned formula. As shown in drawing 3 , the rate of specific histamine isolation was anaclitic to albumen allergen concentration. Moreover, when it became deeper than concentration with allergen concentration, there was also an example used as the bell-like concentration dependence curve to which the rate of histamine isolation falls.

[0071] 4. radioimmunoassay (RIA) -- the histamin release test of cellular segregation and a separation cell was performed by the same approach as 4 of the above-mentioned example using BA312 solid-phase-ized magnetic bead prepared by the approach shown in 3 of the histamine density measurement above-mentioned example by law. the obtained isolation histamine sample -- being related -- radioimmunoassay (RIA) -- histamine concentration was measured in law. 100micro of namely, anti-histamine antibody solutions l which isolated preparatively an isolation histamine sample or 100micro of standard histamine solutions l in the test tube, and were prepared with 0.3% human serum albumin and 125 prepared with 50mM phosphoric-acid buffer saline solution pH 7.0 (assay buffer solution) which contains sodium azide 0.1% I-indicator histamine solution (10 KBq/ml) 100microl, and the assay buffer solution -- in addition, the reaction was performed at 4 degrees C for 2 hours. 500micro of 25% polyethylene-glycol (PEG#6000) solutions l prepared by 2.5% cow gamma globulin solution 100microl prepared with 50mM phosphoric-acid buffer saline solution pH 7.0 (50mMPBS) which contains a sodium azide 0.1%, and 50mMPBS(s) was added to this, and the reaction was performed to it for 10 minutes at 4 degrees C. 1800during 20 minutes xg performed centrifugal separation at 4 degrees C after reaction termination, and the radioactivity of **** was measured. The standard curve obtained by drawing 4 is shown.

[0072]

[Effect of the Invention] Since, as for the monoclonal antibody of this invention, after solid-phase-izing holds antibody activity to support, the chemical mediator isolation by allergen or the anti-IgE antibody load is not checked and isolation of a nonspecific chemical mediator is not induced, the basophilic leucocyte suitable for the specific chemical mediator isolation trial through IgE is separable. Moreover, since the separation approach of the basophilic leucocyte of this invention can separate basophilic leucocyte from a corpuscle simple, it can perform the histamin release test which required conventionally complicated actuation simple. The cell population furthermore obtained by the separation approach of the basophilic leucocyte of this invention is applicable also to the isolation trial for basophilic leucocyte origin chemical mediators which needed the technique conventionally special to handling, such as leukotriene and PAF, simple.

[Translation done.]

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TECHNICAL FIELD

[Industrial Application] This invention is (1). It reacts with basophilic leucocyte and is (2). After the formation of support solid phase holds antibody activity, and it is (3). The specific histamine isolation through IgE of this basophilic leucocyte is not checked, but the monoclonal antibody which does not induce nonspecific histamine isolation of (4) this basophilic leucocyte further, and this monoclonal antibody offer the solid phase-ized monoclonal antibody solid-phase-ized to support. Furthermore, this invention offers the separation approach of the basophilic leucocyte from the body fluid using the hybridoma and this monoclonal antibody which produce this monoclonal antibody, the isolation approach of the chemical mediator from the basophilic leucocyte separated by this separation approach, and the isolation examining method of the chemical mediator from basophilic leucocyte.

[Translation done.]

PRIOR ART

[Description of the Prior Art] The basophilic leucocyte which is a kind of a leucocyte is the target cell of IgE, stores various chemical transmitters (chemical mediator) including a histamine into the granulation, and attracts attention as central existence of an allergic response with the mast cell.

[0003] Usually, the release mechanism of the chemical mediator from basophilic leucocyte is roughly divided into two. A bridge is constructed over the IgE comrade who combined with the receptor on the front face of the film of basophilic leucocyte by allergen and the anti-IgE antibody, degranulation starts one of them by the stimulus, it says that a chemical mediator separates, and isolation by the allergic response is equivalent to this (it is hereafter described as the specific chemical mediator isolation through IgE). Although the release mechanism or meaning are not clarified, another is the isolation produced without constructing a bridge in IgE of a direct basophilic leucocyte film front face, and happens also under an anti-IgE antibody and the nonexistence of allergen in the light of the specific isolation reaction through corresponding IgE (it is hereafter described as nonspecific chemical mediator isolation).

[0004] It is useful to examine the specific chemical mediator isolation which IgE, and a histamin release test occurs as the typical trial. [of the allergosis] [a diagnosis or] [symptoms] Especially, it is the important chemical mediator in which a histamine triggers an I-beam allergic response, and causing many reactions, such as contraction of a bronchial tube smooth muscle and sthenia of blood vessel permeability, is known. By making the gamma E-globulin (IgE) combined with the basophilic leucocyte front face in Homo sapiens peripheral blood through the receptor react with various allergen, a histamin release test is the characteristic detection method using the biological reaction which measures the amount of histamines which separated in connection with this, and, unlike a unique IgE trial (RAST), a skin test, etc. which are other allergen methods of identification, is more useful than that of cause allergen retrieval of an allergic subject as an approach near a clinical manifestation.

[0005] There are an approach of using whole blood, and an approach using a washing leucocyte in the histamin release test using this peripheral blood. Although it is thought that a whole blood method is useful when grasping a patient's allergic state synthetically, other blood serum components other than basophilic leucocyte may affect measurement of histamine isolation. For this reason, when studying the action mechanism of drugs etc. or doing fundamental research of a histamine isolation device, in analyzing the reactivity of exact basophilic leucocyte, it usually uses a washing leucocyte. However, in order to separate a washing leucocyte, after a dextran solution removes an erythrocyte, washing accompanied by centrifugal [2 - 3 times of] is performed, adjusting a white blood cell count further etc. needs to be operated complicated, for this reason, blood volume required for inspection also increases, and there are many difficulties in using as routine laboratory tests.

[0006] Moreover, in the matter emitted, existence of the chemical mediator which shows powerful blood-vessel-permeability sthenia operation and leukocyte migration ability, such as leukotriene and a platelet activating factor (PAF), is known with the degranulation of basophilic leucocyte, and the basophilic leucocyte origin chemical mediator of these versatility is becoming the quality of a fundamental-research object for the purpose of the symptoms analysis of allergy. However, these chemical mediators have many which will be metabolized promptly, when emitted into blood, and especially in case they perform these measurement, they serve as requirements with important handling [a sample] quickly and strictly. Current analysis is very difficult for many of such basophilic leucocyte origin chemical mediators.

[Translation done.]

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EFFECT OF THE INVENTION

[Effect of the Invention] Since, as for the monoclonal antibody of this invention, after solid-phase-izing holds antibody activity to support, the chemical mediator isolation by allergen or the anti-IgE antibody load is not checked and isolation of a nonspecific chemical mediator is not induced, the basophilic leucocyte suitable for the specific chemical mediator isolation trial through IgE is separable. Moreover, since the separation approach of the basophilic leucocyte of this invention can separate basophilic leucocyte from a corpuscle simple, it can perform the histamin release test which required conventionally complicated actuation simple. The cell population furthermore obtained by the separation approach of the basophilic leucocyte of this invention is applicable also to the isolation trial for basophilic leucocyte origin chemical mediators which needed the technique conventionally special to handling, such as leukotriene and PAF, simple.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] As mentioned above, there are specific isolation which minded [of the chemical mediator from basophilic leucocyte] IgE by the allergic response, and the other nonspecific isolation, and it is required for a diagnosis of the allergosis to measure the chemical mediator which separated specifically through IgE. However, in order to perform a histamin release test, without being influenced of a blood serum component as mentioned above and to have to separate a leucocyte, complicated actuation and a lot of blood are required, and it is unsuitable for everyday inspection. Furthermore, since the handling of a sample is complicated also about chemical mediators other than a histamine, a difficult situation performs the present analysis.
[0008] Then, when this invention persons separated basophilic leucocyte out of blood and having been used for the isolation trial of a histamine etc., they inquired by thinking that the above-mentioned problem will be solvable. As separation and a purification method of basophilic leucocyte until now By removing an impurity cell from the sample which raised the consistency of basophilic leucocyte beforehand using the magnetic particle which combined the antibody reacted to cell components other than basophilic leucocyte by the specific gravity centrifuge method P.J. Frederick's and others (P.J.Frederik) method of refining basophilic leucocyte [Journal OBU immuno logical MESOZZU (J.Immunol.Methods) 149 and 207] (1992) etc. -- it is. However, since this approach is not the purification method that separates the basophilic leucocyte itself made into the purpose but a purification method by removing impurity, effectiveness of operability is complicated [an approach] to a bad top.
[0009] on the other hand, many antibodies reacted to the basophilic leucocyte itself are also got to know -- having -- **** -- [-- for example P. -- BARENTO et al. (Valent) and International AKAIBUZU allergy applied immunology (Int.Arch.Allergy Appl.Immunol.) -- 91 and 198 (1990)] Moreover, the monoclonal antibody which shows a specific reaction to Homo sapiens basophilic leucocyte is also reported. [M. P. BOJA et al. (Bodger), brad (Blood) 69 and 1414] (1987) . However, the example which used for the chemical mediator isolation trial of a histamine etc. the Homo sapiens basophilic leucocyte which this basophilic leucocyte reactivity antibody was combined with solid phase support, and the example of a success of the attempt used for separation of Homo sapiens basophilic leucocyte was not known, therefore was separated by such [naturally] approach is not seen, either.
[0010] this fact also considers failure by the loss of basophilic leucocyte reactivity by combining a basophilic leucocyte reactivity antibody with solid phase support etc. -- having (example 1 of the below-mentioned experiment (1)) -- the biggest cause the case where the specific histamine isolation which the basophilic leucocyte isolated preparatively using this basophilic leucocyte reactivity antibody has received the damage, and minded IgE is checked -- [-- for example P. J. Frederick et al., journal OBU immuno logical MESOZZU (J. Immunol.Methods) 149, and 213 (1992), and J.T. Schrader (J.T.Schroeder) ** -- Since nonspecific histamine isolation became remarkably high as compared with the specific histamine isolation through journal OBU immuno logical MESOZZU (J.Immunol.Methods) 133, 269 - 277 (1990)], or IgE, It can consider that it is impossible to be equal to use of the quantum of a histamine isolation reaction etc. in many cases (example 1 of the below-mentioned experiment (2)). P.J. glimpse that Frederick and others refines basophilic leucocyte using cell components other than basophilic leucocyte, and the antibody which reacts -- it is understood as it having been for having taken the roundabout approach also avoiding an above-mentioned fault.

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MEANS

[Means for Solving the Problem] However, in the basophilic leucocyte itself, if separation purification of basophilic leucocyte can be performed using a taste base ball reactivity antibody as a target, achievement of simple and highly precise purification should be attained. Then, this invention persons are monoclonal antibodies, the antibody, i.e., the basophilic leucocyte, which can be used for this purpose, which react, and searched for the monoclonal antibody which has the property of having the reactivity over basophilic leucocyte even after combining with solid phase support, and after association not giving a damage to the basophilic leucocyte concerned with basophilic leucocyte, namely, not having significant effect on the specific histamine isolation and nonspecific histamine isolation through IgE wholeheartedly. Consequently, it succeeded in preparation of the target monoclonal antibody. And after having solid-phase-ized this antibody to support, making it react with body fluid and catching the basophilic leucocyte in this body fluid by this monoclonal antibody, it succeeded in establishing the approach of separating basophilic leucocyte easily, making allergen or an anti-IgE antibody react to the separated basophilic leucocyte, and separating a histamine by removing unreacted body fluid. Furthermore, this invention persons establish the specific histamin release test method through IgE of the basophilic leucocyte separated using this antibody, and came to complete this invention.
[0012] That is, this invention offers the monoclonal antibody which has the following properties.
(1) Basophilic leucocyte and (2) which react (3) to which after the formation of support solid phase holds antibody activity (4) which does not check the specific histamine isolation through IgE of this basophilic leucocyte Nonspecific histamine isolation of this basophilic leucocyte is not induced, [0013] The above which this invention offers (1) (2) (3) And (4) It sets to the monoclonal antibody which has a property, and is (1). It means that this monoclonal antibody reacts reacting with basophilic leucocyte with the surface antigen of basophilic leucocyte.
[0014] Moreover, (2) Even after combining [support solid phase-ization and] this monoclonal antibody with support as holding antibody activity, it means not losing prehension ability of basophilic leucocyte. As this support, although all can be used if a magnetic particle like glass, the granular object made of synthetic resin (bead) or a spherical object (ball), a tube, a plate, and a magnetic bead etc. is the support used when usually solid-phase-izing an antibody for example, it is good to use a magnetic particle preferably.
[0015] The basophilic leucocyte prehension ability of a solid phase-ized antibody is judged by authorizing whether the solid-phase-ized monoclonal antibody combined with basophilic leucocyte, specifically makes a histamine an index, and performs it by the following approaches. First, the solid-phase-ized Homo sapiens basophilic leucocyte and the monoclonal antibody which reacts are made to react to a magnetic bead with Homo sapiens blood. subsequently, the buffer solution for the histamine isolation after collecting beads magnetically and removing supernatant liquid (a calcium chloride --) Add the HEPES buffer solution containing a magnesium chloride etc., and freeze thawing is repeated several times. Histamine gaging system according the total amount of histamines of the supernatant liquid to the known approach, for example, HPLC method, [Y. TSURUTA et al. (Tsuruta), a journal OBU chromatography (J.Chromatogr.) -- 224 and 105 (1981)] (Shimadzu) It measures and the antibody by which the histamine was detected is chosen as an antibody which has basophilic leucocyte prehension ability.
[0016] Moreover, (3) If the specific histamine isolation through IgE of this basophilic leucocyte is not checked, the basophilic leucocyte combined with this monoclonal antibody means maintaining substantially the specific histamine isolation ability through IgE which it originally has in blood. It can check whether it maintains substantially by comparative experiments with monoclonal antibody BA312 discovered in this invention. namely, from the basophilic leucocyte caught by BA312 maintaining the histamine isolation ability which it originally has in blood (example 2 of the below-mentioned experiment) A ******-ed-ized monoclonal antibody is made to react with basophilic leucocyte on solid phase-ized BA312 and these conditions. When the amount of histamines which performed anti-IgE antibody processing and separated under these conditions is 60% or more of that of BA312, the monoclonal antibody concerned judges the obtained both monoclonal antibody joint basophilic leucocyte to be what "does not check the specific histamine isolation through IgE of this basophilic leucocyte." When it comes to the object of this invention, it determines.

[0017] Furthermore, (4) The amount of the nonspecific histamine isolation from the basophilic leucocyte combined with this monoclonal antibody as not inducing nonspecific histamine isolation of this basophilic leucocyte means that it minded IgE that it is 30% or less of the amount of specific histamine isolation.
[0018] The amount of nonspecific histamine isolation can be obtained by measuring the amount of the histamine which separates by adding the hydroxyethyl piperazine-N'-2-ethane-sulfonic-acid (HEPES) buffer solution (buffer solution for histamine isolation) containing the suitable solvent for this basophilic leucocyte, for example, a calcium chloride, a magnesium chloride, a human serum albumin (HSA), etc., and making it react for 10 - 60 minutes at 10-50 degrees C, the known approach, for example, HPLC method.
[0019] The amount of specific histamine isolation can be obtained by measuring the amount of the histamine which separates by making this basophilic leucocyte and the anti-IgE antibody prepared with the buffer solution for histamine isolation through IgE react under the same temperature and reaction time as a top under the same approach and conditions as a top.
[0020] The production approach of the monoclonal antibody of this invention is shown briefly below.
(1) Carry out immunity of the preparation mouse of a hybridoma by Homo sapiens basophilic leucocyte. Immunity is performed by repeating Homo sapiens basophilic leucocyte in the abdominal cavity of a mouse, hypodermically, or a vein several times, and inoculating it into it every several weeks. An antibody forming cell is obtained from the mouse by which immunity was carried out, a myeloma cell is united with this, and a hybridoma is produced. The well of the hybridoma which produces the antibody reacted to the inner Homo sapiens basophilic leucocyte of the obtained hybridoma is chosen.
[0021] After a sheep anti-mouse immunoglobulin antibody combines the antibody to which cloning of the selected hybridoma was carried out, and the hybridoma clone which appeared produced it with the magnetic bead combined beforehand, the property about the prehension ability of the basophilic leucocyte in Homo sapiens blood and the separated histamine isolation of basophilic leucocyte is authorized. Assay of prehension ability can be performed by the above-mentioned approach.
[0022] Assay of the property about histamine isolation of the separated basophilic leucocyte This solid-phase-ized antibody is made to react to a magnetic bead with Homo sapiens blood like assay of prehension ability. After collecting beads magnetically and removing supernatant liquid, according to the above-mentioned approach, the amount of nonspecific histamine isolation and the amount of the specific histamine isolation through IgE are measured. The antibody, i.e., the antibody of the amount of the specific histamine isolation the amount of nonspecific histamine isolation minded IgE which became 10% or less preferably 30% or less, by which the specific histamine isolation through IgE is not checked, and nonspecific histamine isolation is not promoted is chosen.
[0023] Thus, the hybridoma clone which produces the antibody which does not check the specific histamine isolation through IgE of the basophilic leucocyte which does not lose antibody activity, but has the prehension ability of basophilic leucocyte even after combining with support, and was separated, and does not induce nonspecific histamine isolation is chosen, this is cultivated, and monoclonal antibodies are collected.
[0024] Selection of the above-mentioned immunization, a cell fusion method, and syncytium, cloning, etc. can be performed by the well-known usual approach.
[0025] (2) It is in vitro (inch vitro) cultivation or in vivo (invivo) about the hybridoma in which the monoclonal antibody made production selection. It is made to increase by cultivation and a monoclonal antibody is obtained. As in vitro cultivation, a hybridoma can be cultivated until it becomes a growth limitation by the RPMI culture medium (perfect RPMI culture medium) containing fetal calf serum, and a monoclonal antibody can be obtained by collecting the culture supernatants. A hybridoma is transplanted to the abdominal cavity of the mouse which carried out intraperitoneal administration processing of PURISUTEN beforehand as in vivo cultivation, several weeks after, abdomen hypertrophy of a mouse can be confirmed, the ascites can be extracted, and a monoclonal antibody can be obtained for IgG or an IgM fraction from ascites separation and by refining, combining suitably known approaches, such as ammonium sulfate fractionation and a DEAE sepharose column.
[0026] The hybridoma which produces the monoclonal antibodies BA101, BA20, BA135, and BA312 of this invention obtained by the above-mentioned approach From September 10, 1992, to the Fermentation Research Institute in Japan 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken (zip code 305), the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry Respectively "BA101 Shionogi" (Fermentation Research Institute ****** No. 4004, FERM BP-4004), "BA20 Shionogi" (Fermentation Research Institute ****** No. 4005, FERM BP-4005), Based on Budapest Treaty, it ****s as "BA135 Shionogi" (Fermentation Research Institute ****** No. 4006, FERM BP-4006) and "BA312 Shionogi" (Fermentation Research Institute ****** No. 4007, FERM BP-4007). Thus, the monoclonal antibody of this invention was mentioned above although what is produced by the hybridoma deposited, for example was mentioned. (1) - (4) If it is the monoclonal antibody which has the property, all are the range of this invention. Monoclonal antibody BA312 is used preferably.
[0027] The monoclonal antibody of this invention reacts with the leucocyte containing Homo sapiens basophilic

leucocyte, and a human erythrocyte does not react. Furthermore, even after solid-phase-izing to support, the monoclonal antibody of this invention can fully catch basophilic leucocyte, does not induce isolation of the nonspecific chemical mediator of the caught basophilic leucocyte, and does not check the specific chemical mediator isolation through IgE. Moreover, the monoclonal antibody of this invention can be advantageously used as a solid phase-ized monoclonal antibody which is combined with the above support and obtained. It is not limited and especially the method of making it combine with support and making a monoclonal antibody solid-phase-ize is a well-known approach et al., for example, [T. Lee, (Lea) and SUKANJINABIAN journal OBU immunology (Scand.J.Immunol.). 22 and 207 (1985)] can perform. For example, 0-50 degrees C of monoclonal antibodies are made to react preferably at 4-40 degrees C for 30 minutes to 48 hours for 5 minutes to 72 hours with the solid phase support by which the coat was carried out by ANKOTEDDO, the compound with a chemical active group like a tosyl group, or the anti-mouse immunoglobulin.

[0028] This invention is the above and offers the separation approach of basophilic leucocyte which the solid phase-ized monoclonal antibody and body fluid of this invention which are made and obtained are made to react, and is characterized by making it combine with this solid phase-ized monoclonal antibody, and catching the basophilic leucocyte in this body fluid again.

[0029] As this body fluid, although blood, the pituita, tear fluid, saliva, etc. are mentioned, blood is used preferably.

[0030] By the way, G. GODA naks report the approach of combining an antibody with a magnetic particle and separating the target cell. [G. GODA nak et al. (Gaudernack), journal OBU immuno logical method (J.Immunol.Methods) 90, 179] (1986), etc. are known widely. Moreover, the method of refining basophilic leucocyte by removing an impurity cell from the sample which raised the consistency of basophilic leucocyte beforehand using the magnetic particle which combined the antibody reacted to cell components other than basophilic leucocyte by the specific gravity centrifuge method P. It is reported by J. Frederick and others. [P. J. Frederick et al. (Frederic), Journal OBU immuno logical method (J.Immunol.Methods) 149 and 207 (1992)] With this invention There is an essential difference in the part whether to use an antibody [as opposed to cells other than basophilic leucocyte for whether the antibody to basophilic leucocyte is used].

EXAMPLE

[Example] Hereafter, although an example and the example of an experiment explain this invention in more detail, this invention is not limited at all by these examples etc.
1. Production of monoclonal antibody reacted to basophilic leucocyte (1) To two female BALB/c mice of 8 weeks old of immunity, it is 1x106, respectively. The Homo sapiens basophilic leucocyte of an individual is injected intraperitoneally and it is 1x106 3 times per three weeks henceforth, respectively. The Homo sapiens basophilic leucocyte of an individual was injected intraperitoneally.
[0044] (2) Three days after the cell fusion last immunity, the spleen was extracted from two mice and cell suspension was prepared using the RPMI culture medium. This 2x108 The splenic cells of an individual, and 6x107 Myeloma cell NS-1 of an individual was mixed, and in addition, it agitated for 1 more minute after centrifugation, agitating gently polyethylene-glycol (average molecular weight 4000) 1ml 50% to precipitate. For 1 minute was required for 1ml of RPMI culture media, in addition, after adding 1ml similarly, further, for 3 minutes was required and 7ml was added. precipitate is floated after centrifugation to 40ml (perfect RPMI culture medium) of RPMI culture media which contain fetal calf serum 15% -- making -- four 96 hole micro culture plates -- each -- it inoculated 0.1ml into the well at a time, and cultivated at 37 degrees C under existence of 7% carbon dioxide gas. The 0.1 ml perfect RPMI culture medium (HAT medium) containing hypoxanthine 100microM, aminopterin 0.4microM, and thymidine 16microM was added 24 hours after. 2, 3, 5, and eight days [culture initiation and] after, the 0.1 ml culture supernatant was thrown away, and the 0.1 ml HAT medium was added. The 0.1 ml culture supernatant was thrown away on 11 and the 14th, and the 0.1 ml perfect RPMI culture medium (HT culture medium) containing hypoxanthine 100microM and thymidine 16microM was added. The colony of a hybridoma appeared from all wells of a total of 384.
[0045] (3) the selective culture of a hybridoma -- a well -- the inner hybridoma was cultivated by the perfect RPMI culture medium, and the existence of the specific antibody production in the culture supernatant was detected as follows.
[0046] After the dextran physiology salt solution settled 5ml, in addition an erythrocyte to 20ml of Homo sapiens peripheral blood 5%, the leucocyte of supernatant liquid was divided into it. The cell which consists of specific gravity 1.07 to 1.08 was separated from this leucocyte by the discontinuity specific gravity centrifuge method using PARCOR prepared to specific gravity 1.07 and 1.08. About a separation cell, it is the Alcian-blue method. [S.G. Harriet (Harriet) et al., Brad (Blood) 46, 279 (1975)] It dyes, the number of basophilic leucocytes is calculated, and it is 4x105 as basophilic leucocyte. So that it may be set to an individual/ml It prepared with the 10mM hydroxyethyl piperazine-N'-2-ethane-sulfonic-acid (HEPES) buffer solution (pH 7.4) (HA-HEPES buffer solution) containing a 0.8% sodium chloride, 0.037% potassium chloride, and a 0.03% human serum albumin (HSA). To a test tube, it is 2x104 as the number of basophilic leucocytes. The separation cell was poured distributively so that it might become an individual. The culture supernatant of a hybridoma was 50microl Added to this, and it was made to react at 37 degrees C for 1 hour. 50micro of mixed liquor l of the full ORESSEN isothiocyanate (FITC) indicator goat anti-Homo sapiens IgE antibody after the HA-HEPES buffer solution washes [1-time], and a phycoerythrin indicator goat anti-mouse immunoglobulin antibody -- in addition, it was made to react at a room temperature for 1 hour It judged whether the HA-HEPES buffer solution would perform observation by the fluorescence microscope after 1-time washing, and the indicator of the cell by which the FITC indicator was carried out would be carried out by phycoerythrin. Thus, that to which the monoclonal antibody contained in the culture supernatant of a hybridoma reacts was chosen 100 well to the basophilic leucocyte by which the FITC indicator was carried out.
[0047] After carrying out cloning of the hybridoma chosen like the above by limiting dilution, it cultivated to the growth limitation by flask culture, respectively. It was made to react at 4 degrees C for 16 hours in addition to magnetic bead 15mg with which the sheep anti-mouse immunoglobulin antibody has combined 1ml of this culture supernatant beforehand, performing loose churning, and the monoclonal antibody solid phase-ized magnetic bead was obtained. these monoclonal antibody solid phase-ized magnetic beads -- using -- an approach given in the following example 4 -- following -- the basophilic leucocyte prehension ability from Homo sapiens peripheral

blood, and IgE of the separated basophilic leucocyte -- the property about specific histamine isolation and nonspecific histamine isolation was investigated. Consequently, it has the aforementioned property, and hybridoma BA101 Shionogi (FERM BP-4004) which produces the monoclonal antibodies BA101, BA20, BA135, and BA312 suitable for separation of the cell population containing the Homo sapiens basophilic leucocyte of this invention and the histamine isolation test method of a separation cell, BA20 Shionogi (FERM BP-4005), BA135 Shionogi (FERM BP-4006), and BA312 Shionogi (FERM BP-4007) were chosen so that clearly from the example of the after-mentioned experiment.

[0048] Moreover, as a result of investigating the class of the mouse immunoglobulin to which the four above-mentioned sorts of monoclonal antibodies belong, BA20, BA101, and BA135 belonged to IgG1, and it became clear that BA312 belonged to IgM.

[0049] (4) Production of a monoclonal antibody (a) in vitro The cultivation hybridoma was cultivated until it became a growth limitation (1x106 an individual/ml) by the perfect RPMI culture medium, and the culture supernatants were collected.

[0050] (b) in vivo To the BALB/c mouse which carried out intraperitoneal administration processing by 0.5 ml ******** PURISUTEN, it is 5x106. The hybridoma of an individual was transplanted to intraperitoneal. Abdomen hypertrophy of a mouse was confirmed after about three weeks, and the ascites was extracted.

[0051] (5) Purification of a monoclonal antibody (a) The ascites obtained at the protein A affinity chromatography method front process was salted out with the sodium-sulfate solution 18%, and the produced precipitation was dialyzed with this liquid after dissolving in a 0.01M boric-acid buffer saline solution (pH 8.0). 20mg of monoclonal antibodies obtained by the salting-out was melted to 2ml (pH 8.0) of 0.01M boric-acid buffer saline solution, and it was made to stick to the column (1.6x5cm) of protein A-sepharose [Pharmacia Corp. (Pharmacia AB)]. First, the impurity was flowed out by about 50ml (pH 8.0) of 0.01M boric-acid buffer saline solution, subsequently it was eluted by about 100ml (pH 4.0) of 0.01M citric-acid buffer saline solution, and the purification monoclonal antibodies BA20, BA101, and BA135 were obtained.

[0052] (b) 20mg of monoclonal antibodies obtained by the sepharose CL-4B gel chromatography method aforementioned salting-out was melted to 2ml of 0.01M boric-acid buffer saline solution, fractionation was let pass and carried out to the column (1.6x70cm) of sepharose CL-4B [Pharmacia Corp. (PharmaciaAB)], the Maine peak was isolated preparatively, and purification monoclonal antibody BA312 was obtained.

[0053] 2. Solid phase-ized DINA bead to secondary antibody joint bead of monoclonal antibody M-450 The 30 mg-beads/ml suspension of a sheep anti-mouse immunoglobulin [dynal company (Dynal)] was isolated preparatively in 1ml test tube after churning. After collecting beads magnetically and removing supernatant liquid, 1ml of hybridoma culture supernatants was added, and the reaction was performed at 4 degrees C for 16 hours, performing loose churning. the HA-HEPES buffer solution after collecting beads magnetically and removing supernatant liquid -- 4 times and 4 degrees C -- every [a for / 30 minutes] -- it washed performing loose churning and the monoclonal antibody solid phase-ized magnetic bead was obtained. Moreover, 1ml (20microg/(ml)) of purification monoclonal antibodies prepared with the HA-HEPES buffer solution was added instead of the hybridoma culture supernatant, and the monoclonal antibody solid phase-ized magnetic bead was obtained by the same approach as the above.

[0054] 3. Solid phase-ized DINA bead ANKOTEDDO [to ANKOTEDDO bead of monoclonal antibody M-450 (the 30 mg-beads/ml suspension of dynal company (Dynal)] was isolated preparatively in 1ml test tube after churning.) After collecting beads magnetically and removing supernatant liquid, 1ml (0.3 mg/ml) of 0.05M tris-hydrochloric-acid buffer-solution (pH9.5) solutions of BA312 was added, and it agitated well. The reaction was performed at 4 degrees C for 16 hours, performing loose churning. 0.01M which contain 0.5%HSA after collecting beads magnetically and removing supernatant liquid The HEPES buffer solution (pH 7.4) performed washing every 4 times during 5 minutes, the HA-HEPES buffer solution performed washing at 4 degrees C further for 16 hours, and BA312 solid-phase-ized magnetic bead was obtained.

[0055] 4. 40ml of HA-HEPES buffer solutions was added to the cellular segregation by the monoclonal antibody solid phase-ized magnetic bead, and 10ml of healthy human blood liquid which collected blood in histamine isolation reaction EDTA blood collecting tubing, and blood was prepared 20%. The magnetic bead which solid-phase-ized the culture supernatant of hybridoma BA20 Shionogi, BA101 Shionogi, BA135 Shionogi, and BA312 Shionogi, respectively was prepared so that it might be set to 3 mg-beads / ml with the HA-HEPES buffer solution. 20% blood -- a test tube -- every [500microl] -- pouring distributively -- here -- the above BA20, BA101, and BA135 and the formation of BA312 solid phase -- magnetic bead 50microl was added and the reaction was performed for 5 minutes under the loose shaking condition at the room temperature. After collecting beads magnetically and removing supernatant liquid, 500micro of HA-HEPES buffer solutions l performed washing once. After washing termination, a 0.5mM calcium chloride, a 0.25mM magnesium chloride, A 0.1% glucose, a 0.9% sodium chloride, 0.035% sodium bicarbonate, And 20mM(s) containing 0.1%HSA HEPES buffer solution (pH 7.0) (buffer solution for histamine isolation), Or it agitated by adding 500micro (HE-69B) of monoclonal anti-Homo sapiens IgE antibodies l prepared so that it might become in ml and 4microg /with the buffer solution for histamine

isolation. The total amount of histamines of a separation cell made the specimen what repeated freeze thawing 3 times and performed it by the specimen which added the buffer solution for histamine isolation. IgE -- the specific histamine isolation reaction was performed by making the specimen which added the monoclonal anti-Homo sapiens IgE antibody react at 37 degrees C for 1 hour. Moreover, the nonspecific histamine isolation reaction was performed by making the specimen which added the buffer solution for histamine isolation react at 37 degrees C for 1 hour. It agitated after reaction termination, and after carrying out at-long-intervals ** for 1500rpmx 5 minutes, 300micro of supernatant liquid l was isolated preparatively. Measurement of the amount of isolation histamines is a histamine gaging system by the HPLC method. [Y. TSURUTA (Tsuruta) et al., journal OBU chromatography (J.Chromatogr.) 224 and 105] (1981) (Shimadzu) performed.

[0056] As shown in Table 1, when the total amount of histamines of the cell separated with BA20, BA101, BA135, and BA312 solid-phase-ized magnetic bead was measured, it turned out that a histamine is detected in all antibodies and it has the separability of basophilic leucocyte. moreover, it is shown in drawing 1 -- as -- IgE of a separation cell -- the specific rate of histamine isolation -- good -- in addition -- and nonspecific histamine isolation was suppressed to 30% or less as compared with specific histamine isolation.

[0057]

[Table 1]

	BA20	BA101	BA135	BA312	非固相ビーズ
血液検体	++	++	++	++	-

好塩基球の分離能　++：有り　-：なし

[0058] Comparison with an example of experiment 1.CD antibody, and this invention monoclonal antibody (1) Basophilic leucocyte prehension ability P. BARENTO's and others report [P. BARENTO et al. (Valent), International archives allergy applied immunology (Int.Arch.Allergy Appl.Immunol.) It is based on 91 and 198 (1990)]. CD antibody reacted to the antigen well discovered on a basophilic leucocyte front face, i.e., CD9 antibody, (TP82 and NICHIREI CORP.) The antibody solid phase-ized magnetic bead was prepared by the approach shown in 2 of the above-mentioned example using each antibody of a CD11b antibody (BEAR1, immuno tech company), CD13 antibody (MCS2 and NICHIREI CORP.), and CD32 antibody (two E1, immuno tech company). The specific cell population was separated by the same approach as 4 of an example using above-mentioned CD antibody and BA20, and BA312 solid-phase-ized magnetic bead, and the total amount of histamines of the separation cell origin was measured.

[0059] Although good basophilic leucocyte prehension was accepted in CD9 antibody, a CD11b antibody, and BA20 and BA312 as shown in Table 2, separation of basophilic leucocyte was not accepted in CD13 antibody and CD32 antibody.

[0060]

[Table 2]

	CD9	CD11b	CD13	CD32	BA20	BA312
血液検体A	++	++	-	-	++	++
血液検体B	++	++	-	-	++	++

好塩基球捕捉能　++：有り　-：なし

[0061] (2) 1. (1) of the example of the histamine isolation ability above-mentioned experiment of a separation cell Inside of examined CD antibody, CD9 antibody basophilic leucocyte prehension ability was accepted to be, a CD11b antibody, and the histamine isolation ability of the separation cell by each antibody solid phase-ized magnetic bead of BA20 and BA312, That is, three items of the total amount of histamines, the amount of IgE specific isolation histamines, and the amount of nonspecific isolation histamines were measured by the same approach as 4 of the above-mentioned example using the healthy human blood liquid of 20 examples.

[0062] consequently -- while checking separation of basophilic leucocyte by all antibodies -- the difference for every blood specimen -- all the antibodies of a certain thing -- IgE -- specific histamine isolation was accepted.

[0063] In this invention, if the ratio of the amount of the nonspecific histamine isolation through IgE of the basophilic leucocyte caught and separated by the antibody solid-phase-ized in the MAGUNETCHIKKU bead to the amount of specific histamine isolation is 30% or less, it will be thought that this antibody is useful by the chemical

mediator isolation examining method, and it will be especially thought at 10% or less that it is useful. CD9 antibody and a CD11b antibody -- IgE -- since the specimens which show remarkable high nonspecific histamine isolation have occurred frequently as compared with specific histamine isolation, it is shown in Table 3 -- as -- the above-mentioned ratio -- 30% -- large -- exceeding -- **** -- IgE from these separation cells -- it was very difficult to perform analysis about specific histamine isolation. On the other hand, BA312 which is the monoclonal antibody of this invention was extremely excellent in this ratio with 7.5%.

[0064]

[Table 3]

モノクローナル抗体	CD9	CD11b	BA20	BA312
Non-Spec./Spec.(%)	46.9	39.7	22.0	7.5
SD	17.6	28.5	15.1	5.3

Non-Spec. ／Spec. ：ＩｇＥを介した特異的なヒスタミン遊離の量に対する非特異的なヒスタミン遊離の量の比率

SD：標準偏差

[0065] as mentioned above, the rate of basophilic leucocyte prehension -- high -- IgE -- specific histamine isolation -- good -- in addition -- and it was a separation cell by BA20 and BA312 solid-phase-ized magnetic bead that nonspecific histamine isolation is suppressed most. The separation cell by BA312 solid-phase-ized magnetic bead was especially most suitable for performing separation of the basophilic leucocyte from the Homo sapiens blood of this invention, and the histamin release test of this separation cell.

[0066] 2. Comparison examination of the histamin release test by whole blood and the separation cell was performed using whole blood and one histamin release test healthy people volunteer blood by the separation cell. By the approach by whole blood, it collected blood in 10ml of blood in heparin blood collecting tubing first. every [100micro of this blood l] was poured distributively in the test tube, and it stirred by adding the monoclonal anti-Homo sapiens IgE antibody (HE-69B) prepared so that it might be set to 50000, 5000, 500, 50, and 5 ng(s)/ml at this with the buffer solution for histamine isolation, or the buffer solution for histamine isolation 400microl every, respectively (a monoclonal anti-Homo sapiens IgE antibody is set to final concentration 40000, 4000, 400, and 40 and 4 ng(s)/ml). The total amount of histamines made the specimen what repeated freeze thawing 3 times and performed it by the specimen which added the buffer solution for histamine isolation. IgE -- the specific histamine isolation reaction was performed by making the specimen which added the monoclonal anti-Homo sapiens IgE antibody react at 37 degrees C for 1 hour. Moreover, the nonspecific histamine isolation reaction was performed by making the specimen which added the buffer solution for histamine isolation react at 37 degrees C for 1 hour. It stirred after reaction termination, and after carrying out at-long-intervals ** for 1500rpmx 5 minutes, 300micro of supernatant liquid l was isolated preparatively. The histamine gaging system (Shimadzu) by the HPLC method performed measurement of the amount of isolation histamines.

[0067] By the approach by the separation cell, cellular segregation and the histamin release test of a separation cell were performed by the approach of 4 of the above-mentioned example, and the same approach. The monoclonal anti-Homo sapiens IgE antibody (HE-69B) was prepared so that it might be set to 40000, 4000, 400, 40, and 4 ng (s)/ml with the buffer solution for histamine isolation, and it asked for the rate of histamine isolation by this. The rate of specific histamine isolation is computed in a degree type.

[0068]

[Equation 2]

特異的ヒスタミン遊離率（%）＝

$$\frac{\text{IgE 特異的遊離ヒスタミン量} - \text{非特異的遊離ヒスタミン量}}{\text{総ヒスタミン量} - \text{非特異的遊離ヒスタミン量}} \times 100$$

[0069] As shown in drawing 2 , the rate of specific histamine isolation obtained the equivalent result by the approach by whole blood, and the approach by the separation cell. This showed that the separation cell obtained using the monoclonal antibody of this invention was maintaining the histamine isolation ability which it originally has in blood.

[0070] 3. Cellular segregation and the histamin release test of a separation cell were performed by the same approach as 4 of the above-mentioned example using BA312 solid-phase-ized magnetic bead prepared by the approach shown in 3 of the above-mentioned example about two child atopic dermatitis patient blood specimens

whose histamin release test albumens of the separation cell by the clinical specimen are cause allergen. The albumen allergen used for the trial was prepared so that it might be set to 4000, 400, 40, 4, and 0.4 ng/ml with the buffer solution for histamine isolation, and it computed the rate of IgE specific histamine isolation by this according to the above-mentioned formula. As shown in drawing 3 , the rate of specific histamine isolation was anaclitic to albumen allergen concentration. Moreover, when it became deeper than concentration with allergen concentration, there was also an example used as the bell-like concentration dependence curve to which the rate of histamine isolation falls.

[0071] 4. radioimmunoassay (RIA) -- the histamin release test of cellular segregation and a separation cell was performed by the same approach as 4 of the above-mentioned example using BA312 solid-phase-ized magnetic bead prepared by the approach shown in 3 of the histamine density measurement above-mentioned example by law. the obtained isolation histamine sample -- being related -- radioimmunoassay (RIA) -- histamine concentration was measured in law. 100micro of namely, anti-histamine antibody solutions l which isolated preparatively an isolation histamine sample or 100micro of standard histamine solutions l in the test tube, and were prepared with 0.3% human serum albumin and 125 prepared with 50mM phosphoric-acid buffer saline solution pH 7.0 (assay buffer solution) which contains sodium azide 0.1% I-indicator histamine solution (10 KBq/ml) 100microl, and the assay buffer solution -- in addition, the reaction was performed at 4 degrees C for 2 hours. 500micro of 25% polyethylene-glycol (PEG#6000) solutions l prepared by 2.5% cow gamma globulin solution 100microl prepared with 50mM phosphoric-acid buffer saline solution pH 7.0 (50mMPBS) which contains a sodium azide 0.1%, and 50mMPBS(s) was added to this, and the reaction was performed to it for 10 minutes at 4 degrees C. 1800during 20 minutes xg performed centrifugal separation at 4 degrees C after reaction termination, and the radioactivity of **** was measured. The standard curve obtained by drawing 4 is shown.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] They are the total amount of histamines from the basophilic leucocyte divided into the magnetic bead using the monoclonal antibody of solid-phase-ized this invention (white bar graph), the amount (slash bar graph) of the specific histamine isolation through IgE, and the graph that shows the amount (black bar graph) of nonspecific histamine isolation.
[Drawing 2] It is the monoclonal anti-Homo sapiens IgE antibody concentration dependence curve of a histamine isolation reaction using the separation cell by whole blood and BA312 solid-phase-ized magnetic bead.
[Drawing 3] It is the albumen allergen concentration dependence curve of a histamine isolation reaction using the child atopic dermatitis patient blood origin separation cell by BA312 solid-phase-ized magnetic bead.
[Drawing 4] It is the standard curve of the histamine concentration obtained by radioimmunoassay. B/Bo shows % of the joint analyte by which the indicator was carried out.

[Translation done.]

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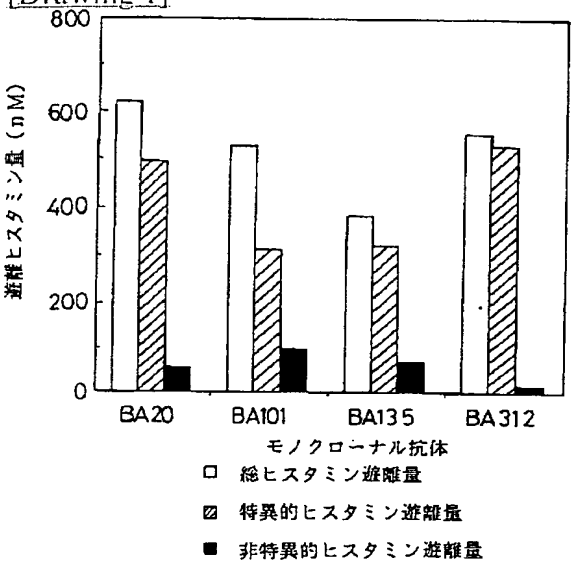
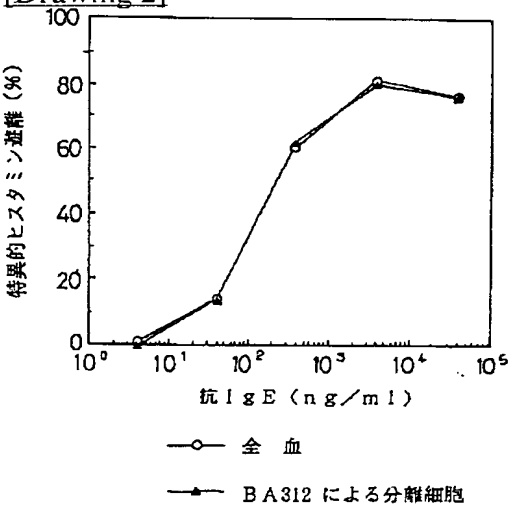
DRAWINGS

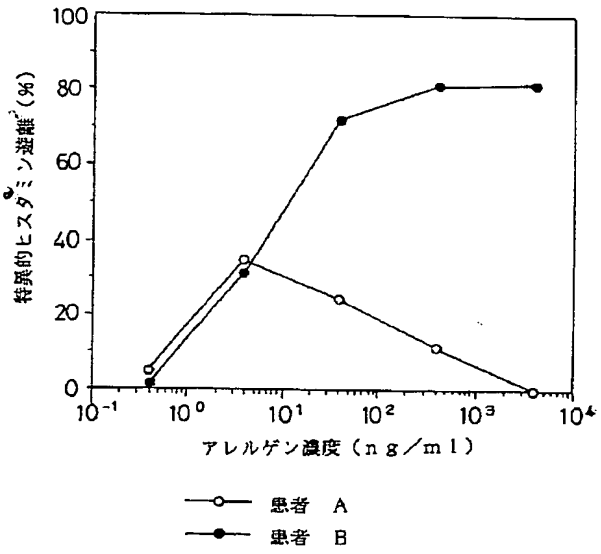
[Drawing 1]



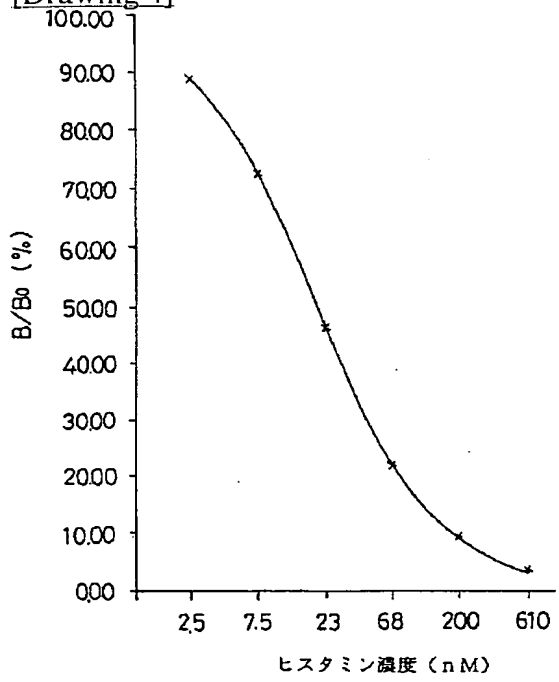
[Drawing 2]



[Drawing 3]



[Drawing 4]



[Translation done.]